

Cucumis metuliferus reduces *Meloidogyne incognita* virulence against the *Mi1.2*
resistance gene in a tomato-melon rotation sequence

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ABSTRACT

BACKGROUND: The susceptible tomato cv. Durinta, ungrafted or grafted onto cv. Aligator resistant rootstock, both followed by the susceptible melon cv. Paloma, ungrafted or grafted onto *Cucumis metuliferus* BGV11135, and in reverse order, were cultivated from 2015 to 2017 in the same plots in a plastic greenhouse, infested or not with *Meloidogyne incognita*. For each crop, the soil nematode densities, galling index, number of eggs per plant and crop yield were determined. Moreover, virulence selection was evaluated in pot experiments. **RESULTS:** In the tomato-melon rotation, the nematode densities increased progressively for the grafted tomato, being higher than for the ungrafted plants at the end of the study; but not so in the melon-tomato rotation. The grafted crops yielded more than the ungrafted ones in the infested plots. Virulence against the *Mi1.2* gene was detected, but not against *C. metuliferus*. Reproduction of *M. incognita* on the resistant tomato was around 120% that on the susceptible cultivar after

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the first grafted tomato crop, but this decreased to just 25% at the end of the experiment.

CONCLUSION: Alternating different resistant plant species suppresses nematode population growth rate and yield losses. However, although do not prevent the selection of virulence, the level was reduced.

Key words: *Cucumis melo*, grafting, resistance durability, root-knot nematode, *Solanum lycopersicum*,

1 INTRODUCTION

Root-knot nematodes (RKN), *Meloidogyne* spp., are the most harmful parasitic nematodes for vegetable crops worldwide¹. Vegetable yield losses caused by RKN under protected cultivation have been estimated to reach maximum values of 88% in cucumber², 62% in tomato³, 39% in zucchini-squash⁴, and 37% in watermelon⁵.

Currently, chemical control, either alone or combined with other methods, is frequently used to manage RKN densities^{6,7}. Nonetheless, the use of pesticides must be reduced in accordance with European directive 2009/128/CE via increased application of natural pest control mechanisms, in line with integrated pest management. Among such natural mechanisms, plant resistance is the most effective, economical and environmentally friendly control method; and it is easy for farmers to use^{8,9}. However, the effectiveness of plant resistance decreases or is lost entirely after repeated cultivation of the same resistance gene or *R*-gene^{10,3}.

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Alternating the *R*-genes in crops via rotation sequences could prevent the selection of RKN populations that are virulent against each gene, and thus improve resistance durability. Unfortunately, there is little diversity among *R*-genes in commercial vegetable cultivars or rootstocks to the most widespread RKN species: *M. arenaria*, *M. incognita* and *M. javanica*. Within solanaceous and cucurbit crops, the most economically important cultivated vegetables worldwide, three *R*-genes can be found in commercial pepper (*Me1*, *Me3* and *N*), and only one in tomato (*Mi1.2*)^{11,12}. Additionally, resistance to RKN in the Solanaceae family has been found in several wild accessions; for example, in *Solanum arcanum*¹³, *S. sisymbirifolium*¹⁴, *S. sparsipilum*¹⁵, and *S. torvum*¹⁶. For cucurbit crops, no cultivars resistant to RKN are commercially available, and they are mostly grafted onto hybrid *Cucurbita* rootstocks that are resistant to fusarium wilt but susceptible to RKN^{17,18,9}. Nonetheless, resistance has also been found in wild accessions of different cucurbit genera: *Cucumis*, including *C. africanus*, *C. anguria*, *C. ficifolius*, *C. metuliferus* and *C. myriocarpus*¹⁹; and *Citrullus*, including *Citrullus lanatus* var. *citroides*²⁰. All these species represent putative germplasm that could be used as commercial rootstocks or in breeding programmes to obtain commercial resistant cultivars.

In the case of *C. metuliferus*, the resistance response to RKN is associated with hindrance of larval development, delayed development from second-stage juveniles (J2) to adults, increased maleness of J2^{21,22}, migration of J2 from the root, differential expression of several genes related to plant defence mechanisms²³, and the appearance of necrotic areas surrounding the nematode²⁴. Rotation sequences including solanaceous

and cucurbits species in protected cultivation are very common, because these crops represent the main source of income for many growers^{25,26,6,7,9}. So, alternating resistant solanaceous cultivars with resistant cucurbitaceous ones could be an efficient way to manage RKN densities by preventing the selection of virulent populations and consequently reducing crop yield losses. *C. metuliferus* is resistant to RKN populations that are (a) virulent against the *Mi1.2* gene, and it is compatible melon rootstock²⁴.

To the best of our knowledge, however, there is no information available on the effect of rotating *C. metuliferus* with RKN-resistant crops on the potential selection of RKN populations that are virulent against both the *Mi1.2* tomato gene and *C. metuliferus*. Selection of RKN for their virulence can be detected by an increase in the final RKN population density on the resistant germplasm, compared to that on the susceptible germplasm, at the end of the crop (P_f), for a given initial RKN density at transplanting (P_i). That is, the RKN population growth rate (the relationship between the rate of multiplication (P_f/P_i) and P_i) on resistant germplasm tends to be similar to that of the susceptible one³. In addition, virulence is tested for by comparing RKN reproduction on resistant versus susceptible germplasm in pot experiments at constant soil temperatures above 28°C, using the field nematode population as an inoculum^{8,10}. Moreover, the reproduction index (RI), that is, the proportion of RKN reproduction on the resistant germplasm compared to that on the susceptible germplasm, allows to estimate the level of plant resistance²⁷ as well as nematode virulence to a given R -gene(s)^{8,10}.

The efficacy of alternating resistant germplasm could be affected by soil temperatures. In the case of the *Mi1.2* gene, its expression may be reduced at soil temperatures over

32°C²⁸, depending on the time spent under these conditions^{29,30}. So, the sequence of the crops in rotation must be considered to select the most suitable for achieving the highest level of nematode suppression and therefore to maximize crop yield without compromising the durability of any resistance gene(s). Thus, the objective of this study was to determine the effect of three-year rotation sequences including tomato and melon, ungrafted or grafted onto RKN-resistant germplasm, on nematode suppression, disease severity, crop yield and putative virulence selection; as well as the optimal sequence of crops in the rotation scheme.

2 MATERIALS AND METHODS

2.1 *Plastic greenhouse experiments*

The experiment was carried out in a 700 m² experimental plastic greenhouse located in Viladecans (Barcelona, Spain) over three growing seasons (2015, 2016 and 2017). The soil was sandy loam with 83.8% sand, 6.7% silt and 9.5% clay; pH 8.7; 1.8% organic matter (w/w); and 0.5 dS m⁻¹ electrical conductivity. The plastic greenhouse was solarized from July to September in 2014. Afterwards, 75% of the soil was infested with the avirulent *Mi1.2* gene isolate Agropolis from *M. incognita* by planting infected tomato cv. Durinta (Seminis Seeds) in October 2014 and harvesting them in February 2015. The tomato plants were obtained from a commercial nursery and were inoculated with 100 eggs and 100 J2 per polystyrene tray cell 7 days before transplanting. The *M. incognita* isolate was obtained in 2010 from roots of the susceptible tomato cv. Durinta,

grown in a plot previously cultivated with susceptible tomato or cucumber, or maintained in black fallow since 2007. The nematode isolate was maintained in susceptible tomato cultivated in pots and identified by the morphology of the perineal pattern and by sequence-characterized amplified region (SCAR) markers³¹. The *Mi1.2* gene and *C. metuliferus* avirulence status of the isolate were determined previously^{3,24}. The remaining 25% of the soil was planted with non-inoculated tomato cv. Durinta, which did not show nematode infection and reproduction at the end of the crop cycle. The experiment consisted of four treatments: i) susceptible tomato cv. Durinta grafted onto the resistant rootstock Aligator (previously PG76) (Gautier seeds) (GT) followed with susceptible melon cv. Paloma (Fitó Seeds) grafted onto the resistant *C. metuliferus* accession BGV11135 from the Institute for Conservation and Improvement of Valencian Agrodiversity (COMAV-UPV) collection (Valencia, Spain) (GM); ii) ungrafted tomato cv. Durinta (T) followed by ungrafted melon cv. Paloma (M); iii) GM-GT; and iv) M-T. Each treatment was cultivated in both *M. incognita* infested and non-infested plots. Crops were grown from March to July and July to November each year in two rotation schemes, tomato-melon (GT-GM, T-M) and melon-tomato (GM-GT, M-T); except in 2017, when only the spring crop of each rotation (March to September) was grown (Fig. 1). Each treatment was replicated 10 times. Individual plots of 3.75 m² consisted of 2.5 m long, containing 4 plants with 0.55 m between each. Plots within a row were spaced 0.9 m, with 1.5 m between rows. Grafted or ungrafted plants were cultivated in the same plot each year to determine the effect of alternating resistant plant species on *M. incognita* densities, disease severity, crop yield and the durability of the

resistance of both the *Mi1.2* tomato gene and *C. metuliferus*. The soil in each plot was prepared separately to avoid cross contamination. Plants were irrigated as needed via a drip irrigation system and fertilized with a solution of NPK (15-5-30) at 31 kg ha⁻¹, and iron chelate and micronutrients at 0.9 kg ha⁻¹. Weeds were removed manually before and during the growing season. Soil temperature and water content were recorded at 1 h intervals with 5TM digital soil probes (Decagon Devices, Inc.) placed at a depth of 15 cm. Tomato and melon fruits were harvested and weighed when they reached commercial standards, and values were expressed as kg plant⁻¹. Initial nematode population densities were determined at transplanting (*Pi*) and finally at the end (*Pf*) of each crop. Soil samples consisted of eight cores taken from the top 30 cm of soil with a 2.5 cm diameter auger, which were mixed and passed through a 4 mm-pore sieve to remove stones and roots. For each experimental plot, J2 were extracted from 500 cm³ of soil using Baermann trays³² and incubated at 27°C±2°C for 1 week. Afterwards, the J2 were collected using a 25 µm aperture screen, counted, and expressed as J2 250 cm⁻³ of soil. At the end of each crop cycle, roots were carefully removed from the soil, washed and weighed, and then the galling index was evaluated on a scale from 0 to 10: 0 = complete and healthy root system, and 10 = plants and roots dead³³. After that, roots of the plants from the same plot were chopped, homogenized, and two 20 g samples of roots were used to determine the number of eggs. The eggs were extracted from roots by maceration in a 10% solution of commercial bleach (40 g L⁻¹ NaOCl) for 10 min³⁴, passed through a 74 µm-aperture sieve to remove root debris, and collected on a 25 µm

sieve, counted and expressed as eggs plant⁻¹. The remaining root samples were used to obtain nematode inoculum to assess putative virulence selection.

The nematode multiplication rate was calculated as $Pf(J2\ 250\ cm^{-3}\ soil + eggs\ plant^{-1}) / Pi\ (J2\ 250\ cm^{-3}\ soil)$, and the relationship between Pf/Pi and Pi was established for each crop and year, in order to determine the putative virulence selection, according to Giné and Sorribas³.

2.2 Virulence selection

The experiments were conducted at the end of each crop cycle. The nematode inoculum consisted of J2 obtained from eggs produced on each plant material: tomato cv. Durinta ungrafted or grafted onto the cv. Aligator rootstock, and melon cv. Paloma ungrafted or grafted onto *C. metuliferus* (Fig. 1). The eggs were extracted from roots by blender maceration in a 5% solution of commercial bleach (40 g L⁻¹ NaOCl) for 5 min³⁴, as previously described. The egg suspension was placed on Baermann trays at 27°C±2°C. Nematodes were collected daily for 7 days using a 25 µm sieve, and stored at 9°C until inoculation. The resistant tomato cv. Monika (Syngenta, Switzerland), the susceptible cv. Durinta, the resistant *C. metuliferus* BGV11135 and the susceptible melon cv. Paloma were used in the experiments. Seeds of *C. metuliferus* were germinated as reported in Expósito et al.²⁴. Tomato seeds were sowed in sterile vermiculite at 25°C±2°C. Seedlings were maintained in a growth chamber at 25°C±2°C with a 16:8 h (light:dark) photoperiod, for a week. Afterwards, the plants were individually transplanted into 200 cm³ pots containing sterile river sand and maintained under the same conditions as before. Plants with three true leaves were singly inoculated with 1 J2

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cm⁻³ of soil. Each plant-subpopulation combination was replicated 10 times. After the first experiment, the avirulent population from the tomato-melon rotation was selected, because no differences were observed between subpopulations from the ungrafted tomato or melon. The plants were maintained in the growth chamber under the same conditions as described previously for 40 days. They were watered as needed and fertilized with a slow release fertilizer (15% N, 9% P₂O₅, 12% K₂O, 2% MgO₂, microelements; Osmocote Plus). Soil temperatures were recorded at 30 min intervals with a PT100 probe (Campbell Scientific Ltd.) inserted into the pots at a depth of 4 cm. At the end of the experiments, roots were carefully washed and weighed. The nematode eggs were extracted from the roots, as previously described. The *RI* for each subpopulation was calculated as the percentage of the number of eggs per plant in the resistant *C. metuliferus* or tomato cv. Monika, in relation to that in the susceptible melon cv. Paloma or tomato cv. Durinta, respectively. The response of the tomato cv. Monika and *C. metuliferus* was categorized according to the *RI* as highly resistant (*RI* < 1%), resistant (1% d *RI* < 10%), moderately resistant (10% d *RI* < 25%), slightly resistant (25% d *RI* < 50%) or susceptible (*RI* e 50%)²⁷. In addition, two experiments were conducted to assess the infectivity, the fecundity and the level of virulence of the subpopulations of the J2 extracted from the soil at the end of the summer crop in 2016, and from those extracted from eggs collected at the end of the spring crop in 2017. The experiments were carried out following the same procedures described previously. The infectivity was considered to be the number of J2 capable of infecting and developing into females laying eggs; and it was expressed as the number of egg masses per plant.

The number of egg masses was counted after dying by submerging the whole root system in a 0.01% solution of erioglaucine for 30 min³⁵. The fecundity was evaluated as the number of eggs laid by each female and expressed as the number of eggs egg mass⁻¹.

2.3 Statistical analysis

Statistical analyses were performed using IBM SPSS statistics v.23 (IBM Corp.). Data for P_i and P_f/P_i were transformed to $\log_{10}(x)$ to linearize them, and subjected to regression analysis for each crop and year, in order to determine the population growth rate. Linear regressions were compared between years for each crop. When no differences were found (intercept and slope $P > 0.05$), the data were pooled to construct a single general model. Regression lines of the grafted and ungrafted crops for each rotation scheme were compared between years, or between general models if no differences were found between years. The galling index and crop yield data were compared between grafted and ungrafted plants for each crop and year; and the crop yield was also compared between infested and non-infested plots. The optimal rotation sequence was determined by comparing the rotation sequences, considering the overall yield of grafted crops in 2015 and 2016, cultivated in infested plots. Comparisons were carried out by means of the non-parametric Wilcoxon signed rank test, as the data did not fit a normal distribution. Data on number of egg masses, eggs plant⁻¹, and eggs egg mass⁻¹ from the virulence selection experiments were compared between resistant and susceptible germplasm, or between nematode subpopulations. All the data were

subjected to the non-parametrical Wilcoxon signed rank test or the Kruskal-Wallis test ($P \leq 0.05$), due to the non-normal distribution of the data.

3 RESULTS

3.1 Plastic greenhouse experiment

The dates of cultivation of each crop, the minimum, maximum and average soil temperatures during cultivation and the range of nematode densities at transplanting each crop are presented in Table 1.

In the tomato-melon rotation scheme, the relationship between P_i and P_f/P_i for ungrafted tomato (T) did not differ between 2015 and 2017 (intercept $P = 0.1122$; slope $P = 0.2992$); however, both these differed from the relationship in 2016 (intercept $P = 0.0002$; slope $P = 0.0127$). For grafted tomato, the relationship between P_i and P_f/P_i differed between 2016 and 2017 (intercept $P < 0.0001$; slope $P = 0.7059$). The population growth rate on ungrafted tomato was higher than on grafted tomato (intercept $P = 0.0008$; slope $P = 0.7156$) in 2016, but it was lower in 2017 (intercept $P < 0.0001$; slope $P = 0.1379$) (Fig. 2A). The grafted tomato showed a lower ($P < 0.05$) galling index than the ungrafted tomato in 2015 and 2016, but a high index ($P < 0.05$) in 2017 (Table 2). The grafted tomato cultivated in infested plots yielded between 64% and 88%, with respect to that in non-infested plots; and between 1.45 and 1.8 times more than the ungrafted tomato in infested plots (Table 2). Regarding the summer melon crop, no differences were found in the population growth rate of the grafted

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melon between 2015 and 2016 (intercept $P = 0.12$; slope $P = 0.8466$). In fact, in melon, only in 2015 were significant regressions found, and the population growth rate differed from that of the grafted melon (intercept $P < 0.0000$; slope $P = 0.2959$) due to the high mortality. A total of 98% of melon plants showed galling index values of 10 at the end of the crop, and this was 40% in 2016 (data not shown). A lower galling index was recorded on grafted than ungrafted melon each year ($P < 0.05$). The grafted melon cultivated in infested plots yielded between 11% and 35% less than that in non-infested plots; but between 8 and 13 times more than the ungrafted melon in infested plots (Table 2).

In the melon-tomato rotation scheme, the relationship between P_i and P_f/P_i for ungrafted and grafted melon did not differ between years (ungrafted melon, 2015 vs 2016: intercept $P = 0.1153$, slope $P = 0.8537$; 2015 vs 2017: intercept $P = 0.4832$, slope $P = 0.7631$; 2016 vs 2017: intercept $P = 0.4589$, slope $P = 0.7818$; grafted melon, 2015 vs 2016: intercept $P = 0.0852$, slope $P = 0.4593$; 2015 vs 2017: intercept $P = 0.3058$, slope $P = 0.9019$; 2016 vs 2017: intercept $P = 0.9856$, slope $P = 0.4894$). The general linear model of the population growth rate for ungrafted melon was higher than for grafted melon (intercept $P < 0.0001$; slope $P = 0.1506$) (Fig. 2C). The grafted melon showed a lower ($P < 0.05$) galling index than the ungrafted melon each year (Table 2). Regarding melon yield, the grafted melon produced 1.3 times more ($P < 0.05$) in infested than non-infested plots in 2015; but did not differ the other years. However, the ungrafted melon cultivated in infested plots produced between 68% and 86% less than in non-infested plots. The grafted melon yielded between 4 and 10.3 times the ungrafted

in infested plots (Table 2). In the following tomato crops, the population growth rate for ungrafted tomato did not differ between years (intercept $P < 0.9828$; slope $P = 0.9592$), but it did for grafted tomato (2015 vs 2016: intercept $P < 0.0001$; slope $P = 0.8600$) being higher in 2016 than in 2015, but lower than for grafted tomato (Fig. 2D). A lower galling index was recorded for grafted than for ungrafted tomato each year. The grafted tomato cultivated in infested plots yielded 20% less than that in non-infested plots in 2016, and did not differ from that of the ungrafted tomato in infested plots (Table 2). The comparison between rotation sequences considering the overall yield of grafted crops cultivated in infested plots in 2015 and 2016 were 15% higher in the melon-tomato rotation sequence than the tomato-melon sequence ($P < 0.05$).

3.2 Virulence selection bioassays

The *RI* for the resistant tomato cv. Monika of the subpopulations from the ungrafted tomato or melon throughout the study ranged from <1% to 5%, corroborating that the tomato cv. Monika was resistant and thus, the nematode subpopulations were avirulent against the *Mi1.2* gene. However, the subpopulations from roots of the first grafted tomato cultivated in both spring-summer and summer-autumn in the plastic greenhouse were fully virulent against the *Mi1.2* gene, according to their *RI* for cv. Monika: *RI* =120% and 118%, respectively. Nonetheless, after cropping the following grafted melon, the *RI* decreased to 39% when cultivated in summer-autumn 2015, and to 14% when cultivated in spring-summer 2016. After that, the *RI* ranged from 13% to 31% (Fig. 3).

The *RI* for *C. metuliferus* ranged from <1% to 13%, irrespective of the plant germplasm in which the subpopulation was developed. So, no virulence selection was observed in this plant germplasm, as it mainly reacted as resistant (1% d *RI* < 10%) over the three years (Fig. 3).

The infectivity and reproduction of the subpopulations obtained from soil after cropping grafted melon or grafted tomato in 2016 were higher ($P < 0.05$) than those of the subpopulation obtained after cropping ungrafted tomato. Nonetheless, the fecundity of the subpopulation obtained after cropping ungrafted tomato was higher than after cropping grafted melon on the resistant tomato cv. Monika. For the susceptible tomato cv. Durinta, the reproduction of the subpopulation after cropping grafted melon was lower than after cropping ungrafted tomato (Table 3). The infectivity, reproduction and fecundity of the nematode subpopulation obtained from grafted tomato roots at the end of the crop in 2017 were lower than the ungrafted tomato subpopulation on the susceptible cv. Durinta ($P < 0.05$). Moreover, the reproduction and fecundity of the subpopulation from grafted tomato were also lower ($P < 0.05$) than those of the subpopulation from ungrafted tomato, on melon cv. Paloma (Table 4).

4 DISCUSSION

The management of RKN is a challenge in intensive horticulture in which crop yield losses can be very important for farm economies. The use of plant resistance is an easy environmentally friendly way to suppress the nematode population growth and has a

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high benefit-to-cost ratio. Nonetheless, this strategy must be used correctly to avoid the selection of virulent nematode populations. The selection of *Mil.2* virulent populations due the reiterative use of resistant germplasm has been reported previously^{36,37,38,3}, and it has become an important problem, as shown by the increasing frequency of virulent RKN populations in commercial areas in recent years^{39,40,41}. Thus, it is very important to include different *R*-genes, because the overlapping of signalling and the recognition of the resistance pathways may result in cross-selection⁴². Along these lines, our working hypothesis was that alternating crops of two different resistant plant species can prevent the selection of virulence against each *R*-gene(s) thereby improving their durability. However, the results of this study have shown that this strategy is not enough to prevent the selection of virulence against one of them; but it does contribute to reducing disease severity and to improving crop yields.

The resistant cv. Aligator rootstock selected an *M. incognita* population with virulence against the *Mil.2* gene after the first tomato crop, irrespective of the crop season. This tomato rootstock was previously reported to be highly resistant in pot experiments and also after being cultivated for one season (March to July) in a plastic greenhouse^{43,38}. Nonetheless, the Aligator rootstock selected a virulent *M. javanica* population in plastic greenhouse experiments after being repeatedly cultivated for three seasons in the same plots¹⁰. This virulence selection was corroborated in pot experiments that show a progressive increase in the level of virulence, year by year, resulting in the resistance being overcome before the third tomato crop ($RI = 90$). Virulence selection is subject to different factors and can be progressive^{10,3}, or occur suddenly^{43,44,45}. Acquired virulence

is a genetically inherited and stable character⁴⁶, but it probably needs a minimum amount of continuous exposure to the resistant germplasm to become fixed in the population. Otherwise, if the population is not continuously exposed, the level of virulence of the population may decrease to a certain intermediate level, as observed with the inclusion of *C. metuliferus* in the rotation scheme. It is accepted that the acquisition of virulent status brings about changes in the fitness of the nematode population with respect to other susceptible plant hosts, compared to avirulent nematodes^{47,48}. The infectivity, reproduction and fecundity fitness of the subpopulation selected with *Mil.2* virulence against the susceptible tomato and melon were reduced with respect to the avirulent subpopulation after the third grafted tomato crop, but not after the second. This indicates that a minimum of three resistant tomato crops are needed to affect the fitness of the intermediate virulent population selected. So, in a nematode population in which (a)virulent individuals coexists, virulence could be counter-selected in susceptible germplasm⁴⁸. Thus, including some more resistant plant species in the rotation scheme alone, or alternating with susceptible ones in order to increase the time elapsed between two crops with the same *R*-gene, could prevent virulence selection. However, even if it does not, virulence could not be fixed in the nematode population and the frequency of virulent individuals would decrease over time. In fact, rotation sequences including resistant and susceptible crops have been proposed as a strategy to reduce the level of virulence and to reduce crop yield losses^{49,50}. Other strategies to manage the emergence of virulent populations have been reported, such as pyramiding *R*-genes. For example, pepper germplasm containing both

Me1 and *Me3* resistance genes pyramided, totally suppressed the emergence of virulent isolates under both laboratory and field conditions⁵¹. Similar results were reported with potato germplasm containing the *GpaIV_{adg}* and *Gpa5* genes pyramided, in which fewer *Globodera pallida* cysts developed than in genotypes carrying each single gene⁵². Regarding tomato, several single dominant *R*-genes that are also resistant against *Mil.2*-virulent RKN populations and stable at high soil temperatures (32°C) have been identified and mapped in different chromosomes⁵³. Such genes could be pyramided in order to obtain stronger and durable resistance in tomato. Similarly, transplanting plants primed by microorganisms which express faster and stronger resistance against RKN⁵⁴ could reduce virulence selection. In addition, the inclusion of other practices in the rotation sequence, before the selection of virulent populations, such as the use of resistant plants or other plant species as a trap cover crop⁵⁵, soil solarization or biofumigation⁵⁶, could also avoid the virulence selection due to the reduced level of nematode infestation of the soil.

In this study, intermittent soil temperatures over 28°C were registered at the end of the spring crop and at the beginning of the summer crop; but the possibility that this triggered the breaking of the resistance is ruled out in accordance with previous work²⁹. High soil temperatures could help the nematode to breakdown the *Mil.2* gene, but this is not plausible as the nematode subpopulations obtained from roots after the first susceptible crop or *C. metuliferus*, which were similarly affected by these high soil temperatures, did not show an increase of *RI* in pot experiments at soil temperatures below 28°C. In addition, the lack of resistance induced by exposure to high soil

temperature is reversed over time, regardless of additional exposure and nematode infection³⁰.

5 CONCLUSIONS

Alternating crops of different resistant plant species suppress nematode population growth rate and crop yield losses. Moreover, although this strategy does not prevent virulence selection, the resultant level of virulence is reduced.

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Figure 1. A: Rotation schemes for 2015, 2016 and 2017 for the tomato-melon (GT-GM;T-M) or melon-tomato (GM-GT;M-T) including susceptible tomato (T) and susceptible melon (M) ungrafted or grafted onto the resistant tomato rootstock cv. Aligator (GT) or resistant *Cucumis metuliferus* (GM) accession BGV11135 respectively in a plastic greenhouse infested with *Meloidogyne incognita* to determine the nematode suppression, disease severity and crop yield. B: Pot experiments conducted with the subpopulations extracted after each crop of the rotation scheme to determine the putative selection of virulence.

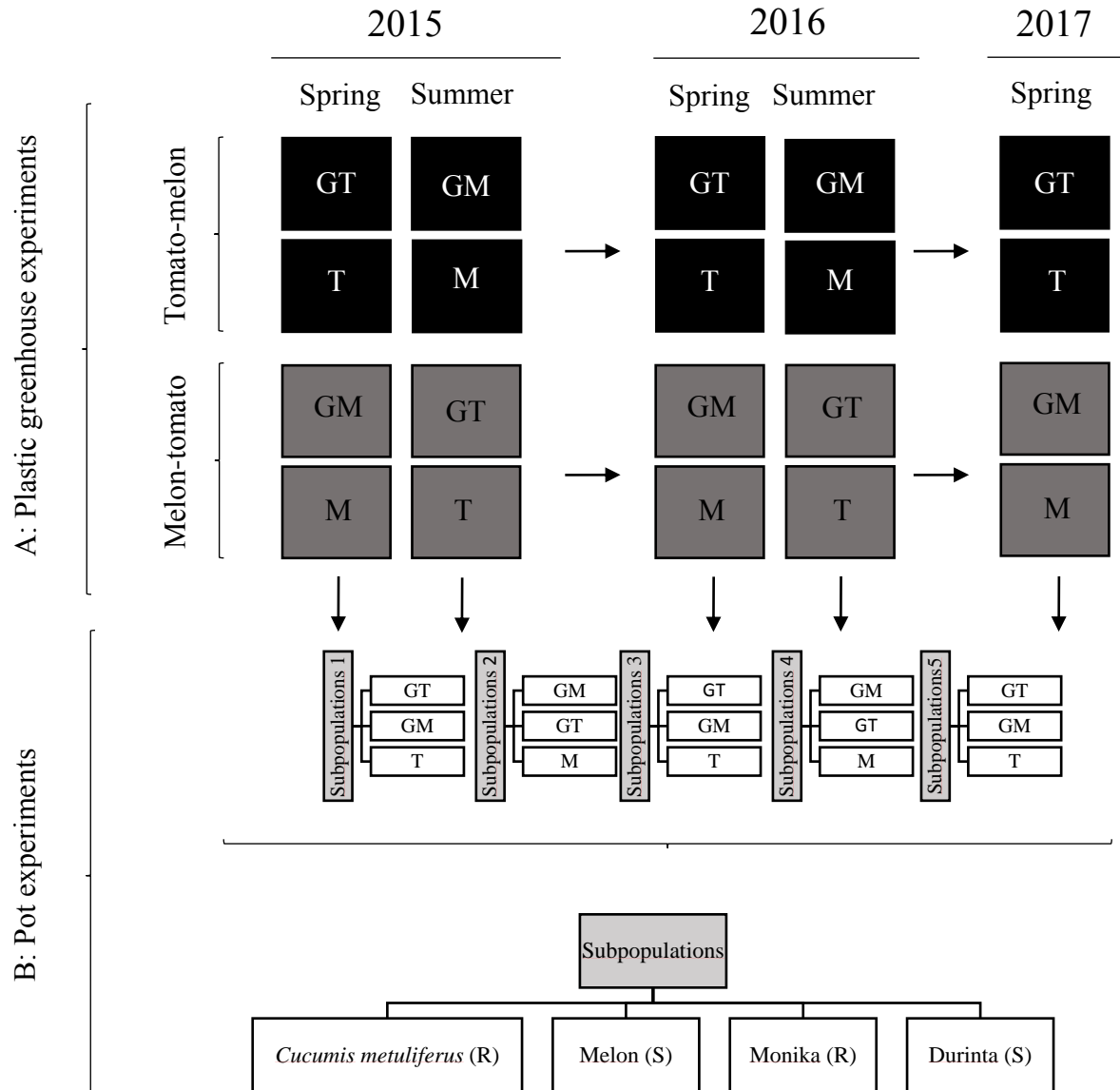
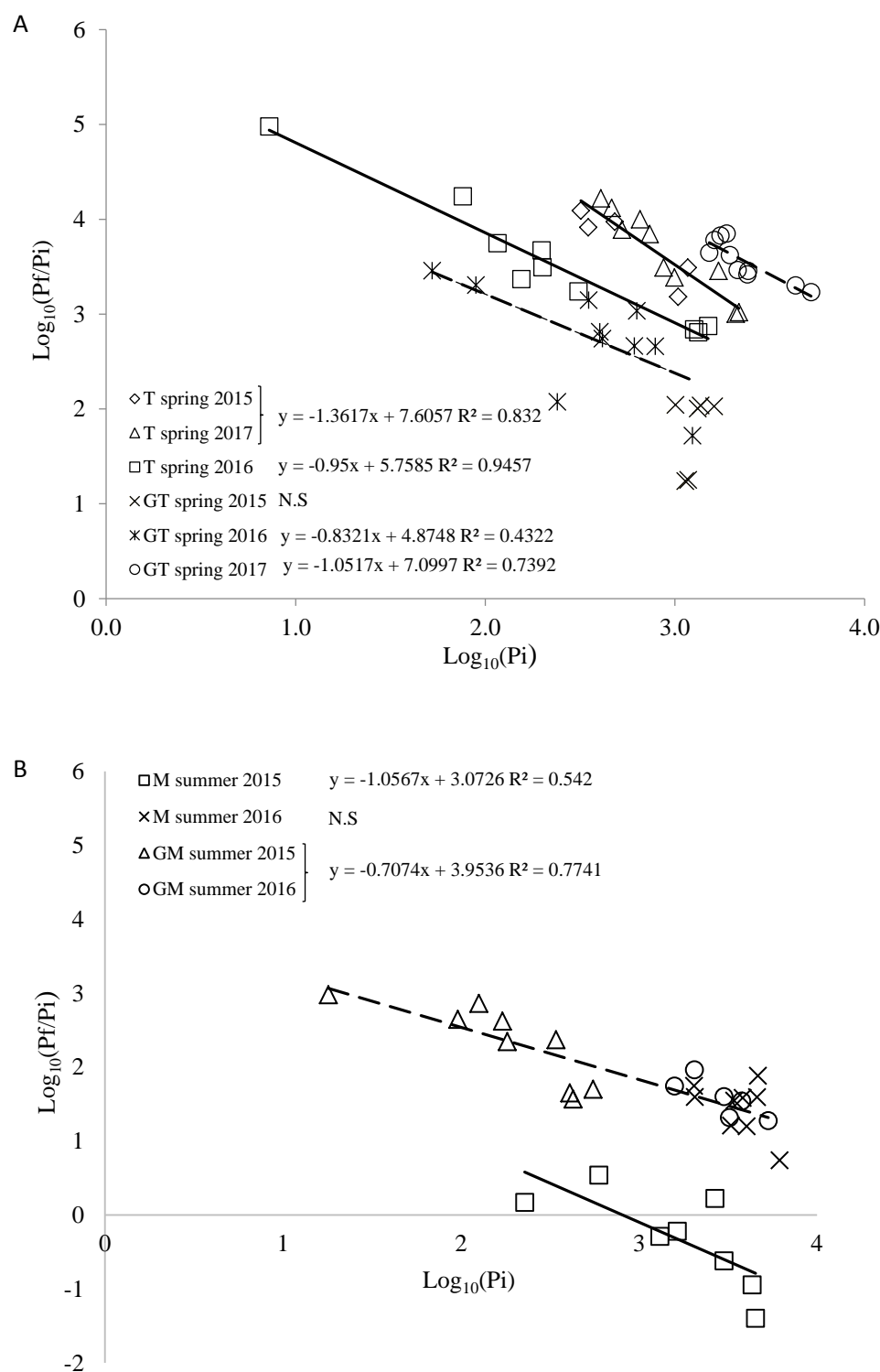


Figure 2. Relationship between the *Meloidogyne incognita* nematode reproduction rate (Pf/Pi) and the population densities at transplanting (Pi) for the susceptible tomato cv. Durinta, ungrafted (T) or grafted onto the resistant tomato rootstock cv. Aligator (GT), and for the susceptible melon cv. Paloma ungrafted (M) or grafted onto the resistant *Cucumis metuliferus* accession BGV11135 (GM) cultivated in a plastic greenhouse during 2015, 2016 and 2017 in a tomato-melon (A and B) or melon-tomato (C and D) rotation scheme. N.S: Not significant.



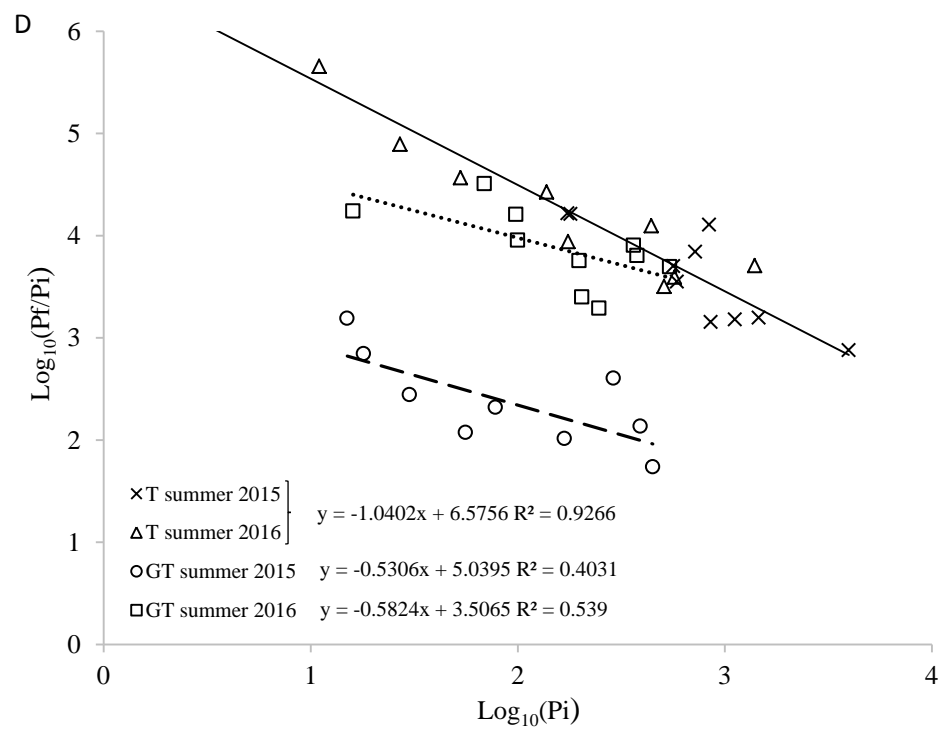
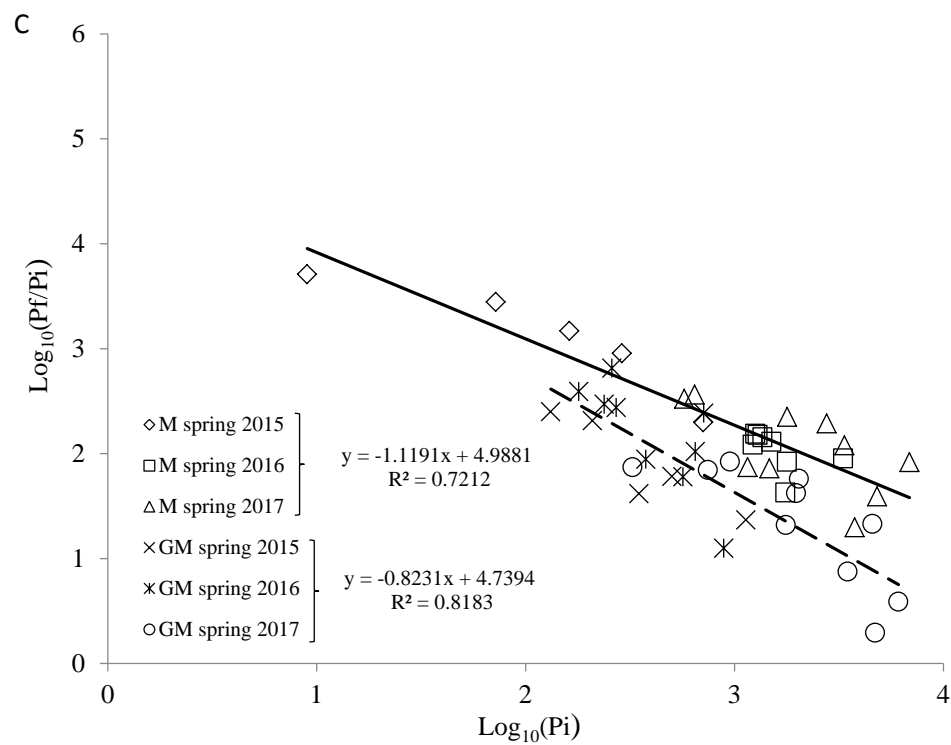


Figure 3. Reproduction index (RI: percentage of the eggs plant⁻¹ produced in the resistant germplasm respect those produced in the susceptible germplasm), of the *Meloidogyne incognita* subpopulations obtained from roots of the susceptible tomato cv. Durinta, ungrafted (T) or grafted onto the resistant tomato rootstock cv. Aligator (GT) and susceptible melon cv. Paloma, ungrafted (M) or grafted onto the resistant *Cucumis metuliferus* accession BGV11135 (GM) cultivated in a plastic greenhouse in 2015, 2016 and 2017 in a tomato-melon (GT-GM;T-M) or melon-tomato (GM-GT; M-T) rotation sequence.

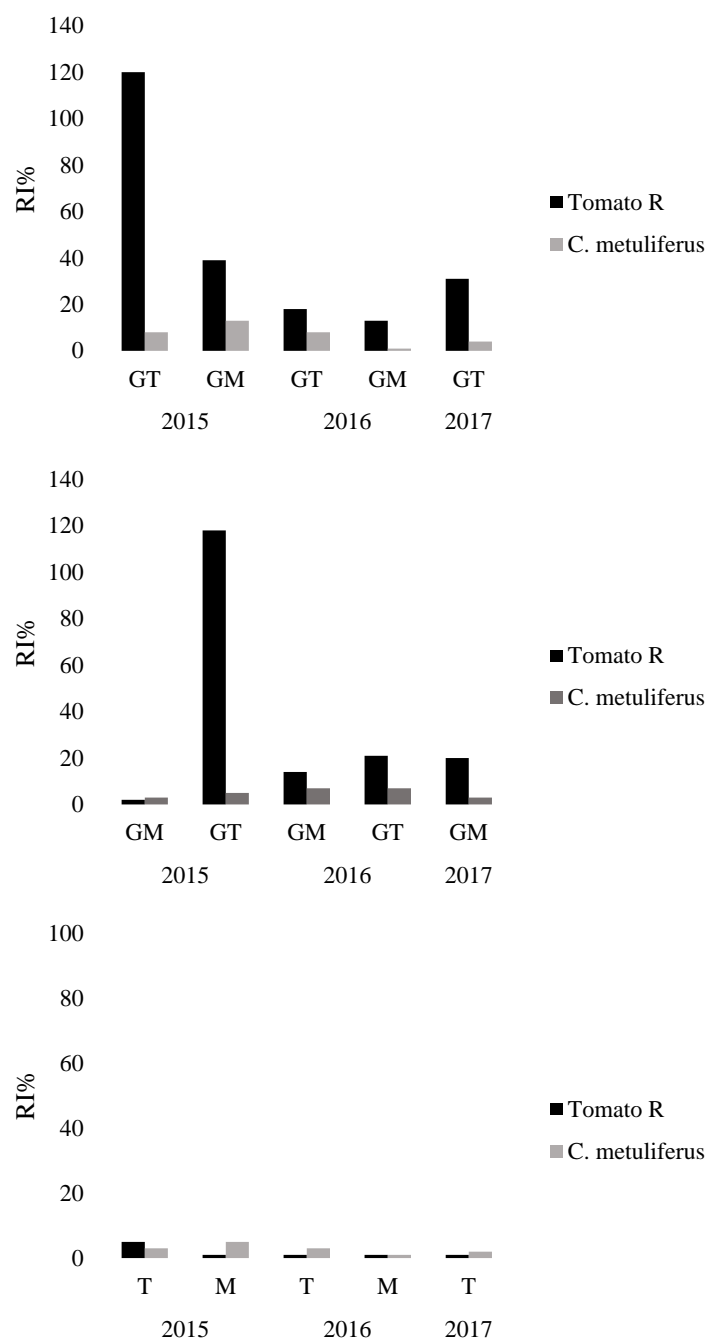


Table 1. Rotation sequence, cultivation dates, soil temperatures and nematode density ranges at transplanting (*Pi*) the ungrafted susceptible tomato cv. Durinta (T) or grafted onto the resistant tomato rootstock cv. Aligator (GT), and the ungrafted susceptible melon cv. Paloma (M) or grafted onto the resistant *Cucumis metuliferus* accession BGV11135 (GM) cultivated in a plastic greenhouse infested with *M. incognita* in 2015, 2016 and 2017

Rotation sequence	Year	Crop	Dates		Soil T (°C)			<i>Pi</i> range (J2 250cm ⁻³)
			Start	End	Min	Max	Av	
Tomato-melon	2015	GT/T	24/3	16/7	17.6	31.9	24.1	0-1611
		GM/M	22/7	26/10	18.3	30.5	24.2	0-4438
	2016	GT/T	15/3	21/7	13.1	29.4	22.1	0-1496
		GM/M	22/7	26/10	18.4	30.5	25.2	0-4657
	2017	GT/T	19/4	12/9	13.8	29.8	24	0-5222
Melon-tomato	2015	GM/M	24/3	16/7	17.6	31.9	24.1	0-1134
		GT/T	22/7	29/10	18.1	30.5	24.1	0-3970
	2016	GM/M	20/4	26/7	14	30	22.5	0-3312
		GT/T	27/7	7/11	17.1	30.6	25.1	0-1395
	2017	GM/M	5/4	28/8	13.1	29.8	24.6	0-6680

Table 2. Gallings index (GI) and yield in the rotation sequence tomato-melon (GT-GM;T-M) and melon-tomato (GM-GT;M-T) of susceptible tomato cv. Durinta, ungrafted (T) or grafted onto the resistant tomato rootstock cv. Aligator (GT) and susceptible melon cv. Paloma, ungrafted (M) or grafted onto the resistant *Cucumis metuliferus* BGV11135 (GM) cultivated in *Meloidogyne incognita* infested or non-infested plots in a plastic greenhouse for three years.

Rotation sequence	Year	Season	Crop	GI [†]	Yield (kg plant ⁻¹)	
					Infested	Non-infested
Tomato-melon	2015	Spring	GT	2 ± 0.2*	3.6 ± 0.2 *b	4.1 ± 0.1 a
			T	8.2 ± 0.1	2 ± 0.2 b	4.4 ± 0.2 a
		Summer	GM	4.3 ± 0.4*	1.3 ± 0.1 *a	2 ± 0.4 a
			M	9.9 ± 0.1	0.1 ± 0.1 b	2.1 ± 0.4 a
	2016	Spring	GT	3.9 ± 0.1*	2.7 ± 0.2 *b	3.7 ± 0.2 *a
			T	6 ± 0.2	1.7 ± 0.2 b	2.7 ± 0.2 a
		Summer	GM	4.6 ± 0.8*	0.8 ± 0.2 *a	0.9 ± 0.1 a
			M	8.2 ± 0.4	0.1 ± 0.1	NA [‡]
	2017	Spring	GT	7.1 ± 0.3*	2.9 ± 0.2 *b	4.5 ± 0.2 a
			T	6.5 ± 0.1	2 ± 0.2	NA [‡]
	Melon-tomato	2015	GM	4.1 ± 0.2*	3.2 ± 0.3 *a	2.5 ± 0.2 b
			M	8.7 ± 0.2	0.8 ± 0.1 b	2.5 ± 0.2 a
		Summer	GT	1.9 ± 0.2*	2 ± 0.2 *a	2.4 ± 0.2 a
			T	7.2 ± 0.2	0.8 ± 0.1 b	2.1 ± 0.2 a
		2016	GM	3.3 ± 0.2*	2 ± 0.2 *a	1.7 ± 0.2 a
			M	5.6 ± 0.3	0.2 ± 0.1 b	1.4 ± 0.1 a
		Summer	GT	5 ± 0.3*	1.6 ± 0.1 b	2 ± 0.2 a
			T	5.9 ± 0.2	1.6 ± 0.1	NA [‡]
	2017	Spring	GM	5.1 ± 0.3*	3.1 ± 0.3 *a	3.4 ± 0.3 a
			M	6.1 ± 0.2	0.3 ± 0.1	NA [‡]

Data are mean of 40 plants ± standard error. Values followed by * are different between grafted and ungrafted plants according to the Wilcoxon signed rank test ($P<0.05$). Values of yield in the same row followed by the same letter are not different according to the Wilcoxon signed rank test ($P<0.05$).

[†]GI: Gallings index (Zeck, 1971)

[‡]NA: Not available, due to cross contamination.

Table 3. Number of egg masses plant⁻¹, eggs plant⁻¹ and eggs egg mass⁻¹ produced on the resistant tomato cv. Monika (R) and the susceptible cv. Durinta (S) in 200cm³ pot experiments inoculated with 1J2 cm⁻³ of the *Meloidogyne incognita* subpopulations obtained from soil after cropping grafted tomato (GT), grafted melon (GM) or tomato (T) in 2016.

Cultivar	Egg masses plant ⁻¹			Eggs plant ⁻¹ (x100)			Eggs Egg mass ⁻¹		
	GT	GM	T	GT	GM [†]	T	GT	GM	T
Monika (R)	29 ± 3 a	32 ± 3 a	9 ± 1 b	195 ± 19 a	161 ± 21 a	85 ± 15 b	693 ± 46 ab	531 ± 63 b	904 ± 58 a
Durinta (S)	102 ± 8 a*	76 ± 6 b*	96 ± 6 ab*	1124 ± 104 a*	433 ± 46 b*	743 ± 70 a*	1240 ± 205 a*	560 ± 38 b	790 ± 77 ab

Data are mean ± standard error of 16 replicates. Values of the same parameter in the same row followed by different letters are significantly different according to the Kruskal-Wallis test ($P < 0.05$). Values of the same column followed by * are different according to the Wilcoxon signed rank test ($P < 0.05$).

[†]GT: subpopulation from the melon-tomato rotation scheme, GM-GT-GM-GT; T: subpopulation from the melon-tomato rotation scheme, M-T-M-T; GM: subpopulation from the tomato-melon rotation scheme, GT-GM-GT-GM;

Table 4. Number of egg masses plant⁻¹, eggs plant⁻¹ and eggs egg mass⁻¹ produced on the resistant tomato cv. Monika (R), the susceptible cv. Durinta (S), the resistant *Cucumis metuliferus* BGV11135 (R), and the susceptible melon cv. Paloma (S) in 200cm³ pot experiments inoculated with 1J2 cm⁻³ of the *Meloidogyne incognita* subpopulations obtained from roots after cropping grafted tomato (GT), grafted melon (GM) or tomato (T) in 2017.

Plant	Egg masses plant ⁻¹			Eggs plant ⁻¹ (x100)			Eggs Egg mass ⁻¹		
	GT	GM	T	GT [†]	GM	T	GT	GM	T
Monika (R)	14 ± 1 a	16 ± 2 a	1 ± 0 b	65 ± 9 a	126 ± 21 a	4 ± 1 b	454 ± 41 b	748 ± 63 a	288 ± 49 b
Durinta (S)	40 ± 4 b*	74 ± 7 a*	77 ± 7 a*	212 ± 26 b*	619 ± 58 a*	873 ± 71 a*	545 ± 52 b	839 ± 55 ab	1211 ± 93 a*
<i>C. metuliferus</i> (R)	4 ± 1 b	6 ± 1 a	6 ± 1 a	20 ± 4 a	20 ± 4 a	17 ± 3 a	418 ± 84 a	355 ± 47 a	334 ± 49 a
Melon (S)	52 ± 3 b*	72 ± 6 a*	67 ± 6 ab*	439 ± 27 b*	721 ± 57 a*	1003 ± 53 a*	851 ± 57 b*	1040 ± 84 b*	1617 ± 151 a*

Data are mean ± standard error of 16 replicates. Values of the same parameter in the same row followed by different letters are significantly different according to the Kruskal-Wallis test ($P < 0.05$). Values of the same column and crop followed by * are different according to the Wilcoxon signed rank test ($P < 0.05$).

[†]GT: subpopulation from tomato-melon rotation GT-GM-GT-GM-GT; GM: subpopulation from the melon-tomato rotation scheme, GM-GT-GM-GT-GM; T: subpopulation from the tomato-melon rotation scheme, T-M-T-M-T